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Studies on the Histology and Histopathology of the Rainbow Trout, *Salmo gairdneri irideus*. II. Effects of Induced Inflammation and Cortisone Treatment on the Digestive Organs[1]

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(Plates I & II)

THIS study was undertaken to determine whether physiological responses to induced inflammation in a poikilothermic vertebrate such as the trout are similar to those found in common laboratory animals. Responses of the digestive tract tissues, particularly those of the stomach and intestines, were studied following chemically and physically induced inflammation. Effects of cortisone on inflammation and wound healing were also determined. The histology of the digestive organs under such experimental conditions was compared with the normal histology previously described (Weinreb & Bilstad, 1955).

Irritants used in different mammals as a means of producing sterile inflammation have included turpentine (Menkin, 1940.1; Cartwright *et al.*, 1951; Shapiro *et al.*, 1951; Moon & Tershakovec, 1951; Spain *et al.*, 1952), croton oil (Clark & Clark, 1920; Michael & Whorton, 1951) and hot water (Menkin, 1933). The relation between the leukocytes, reticulo-endothelial system, and adrenal cortex following turpentine administration was discussed by Cartwright and co-workers (1951).

The influence of various hormones on inflammation has been reported. The effects of adrenal cortical extract were compared with those of other steroid hormones (Menkin, 1942, 1951.1, 1951.2), and the inhibitory effect of cortisone described (Michael & Whorton, 1951; Shapiro *et al.*, 1951; Spain *et al.*, 1952; Rebuck & Mellinger, 1953). Responses to large doses of both cortisone and ACTH were also determined (Robinson & Smith, 1953). The inhibitory effect of steroid hormones and ACTH on granulation tissue and wound healing has been reported by Taubenhaus (1953), Taubenhaus and co-workers (1949, 1952), Baker & Ingle (1948), Castor & Baker (1950) and Baker & Whitaker (1950).

Almost all of the work cited above refers to homoiothermic animals, and few detailed studies have been reported for poikilothermic vertebrates.

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Materials and Methods

Inflammation was initiated chemically by means of intraperitoneal injections of turpentine, and physically by surgical trauma. Four to six rainbow trout were used for each experiment. The animals were injected as described previously (Weinreb, 1958), and tissue was removed immediately following collection of blood samples, at the time intervals indicated. Samples of cardiac stomach and ascending intestine, at the level of the pyloric caeca and pancreas, were excised. Tissues were fixed in Helly's solution and stained with hematoxylin-eosin, hematoxylin - eosin - azure and periodic acid - Schiff

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(PAS) reagent. Two controls were used with PAS, namely salivary digestion and omission of the oxidizing agent. The hematoxylin-eosin-azure technic was that outlined by Yokoyama (1947). The latter procedure was substituted for H & E as the technic of choice, because of its greater differentiation of blood elements in inflammation.

Turpentine-injected trout were also given cortisone, as described previously (Weinreb, 1958). An average of four trout were used per experiment, and sections of stomach and intestine were excised. In addition, one animal received 0.4 cc. Thorotrast (Heyden Chemical Corp., New York) followed by turpentine injection at 24 hours and sacrificed at 30 hours. Phagocytic activity coincident with inflammation was thus compared with similar activity in the normal animal.

Surgical trauma was induced by an incision in the descending intestine. The animal was anaesthesized in dilute urethane and a small incision made in the ventral body wall just anterior to the pelvic fin, at the level of the spleen, after taking precautions to prevent drying. After lifting the intestinal limb through the previous incision, a small cut was made in the intestinal wall. The intestine was carefully replaced and the adominal incision closed by a skin clamp. The entire operation lasted but a few minutes and the trout resumed normal swimming upon return to the tank. The trout were sacrificed and the region of the intestinal incision excised after 12, 24, 48 and 72 hours. The 12- and 24-hour groups each consisted of 6 trout, half of which had been given injections of 1 mg./cc. cortisone 24 hours earlier. Two trout were maintained 48 hours and one for 72 hours. Tissues were fixed in Helly's solution and stained with hematoxylin-eosin-azure and PAS.

Results

Intraperitoneally - injected turpentine caused distress to some of the trout, resulting in difficult respiration and sluggishness. Animals exhibiting increased weakness within one to two hours constituted part of the early mortality group. These animals all had pale gills, attributable to shunting of blood to the sites of injection and inflammation, as well as shock-induced constriction of branchial vessels.

The strong odor of turpentine was prominent in the body fluid and tissues at autopsy. More than usual amounts of blood-tinged fluid were found in the body cavity, accompanied by a whitening and loss of firmness of the fat bodies around the stomach and intestine. Although the viscera appeared paler, there was dilation of the blood vessels of the visceral peritoneum and inflammation of the parietal peritoneum. The lumen of the stomach contained a white fluid. A thicker yellow-orange fluid was found in the intestine; its color probably was the result of increased bile content.

Trout injected with cortisone and turpentine concurrently, exhibited similar distress. Animals which had been injected with cortisone 24 hours previously showed less effect from the turpentine. In addition, the peritoneum and viscera of trout pretreated with cortisone showed almost no change at autopsy, while the former group exhibited gross changes to a lesser degree similar to those in animals given turpentine alone.

Marked histological changes were observed. Lesions were most pronounced in the cardiac stomach and were also evident in the intestine, caeca and pancreas. Microscopic lesions in the stomach were noted as early as one hour following turpentine injection. The submucosa exhibited edema, hyperemia and fibroblastic proliferation. The surface epithelium was vacuolated and granular, while the serous cells of the cardiac glands showed an increase in zymogen granules and secretory activity. Edema, dilation and congestion of blood vessels, with endothelial swelling, continued throughout the first 6 hours. After 3 hours an increase in the number of cells and the amount of intracellular granulation was observed in the stratum granulosum. The lumen was lined by an exudate containing blood cells, bacteria and debris. A purulent exudate covered the serosa. The reaction was similar after 5 hours, with additional leukocytic and macrophagic infiltration of the submucosa.

The increase in fibroblasts and granule cells, the latter layer being more than double (5-6 cells deep) the normal thickness, was most marked after 7 hours (Pl. I, Fig. 1). This was accompanied by an increase in collagen and thickening of the mucosal basement membranes. After 7 hours the edema of the submucosa and muscle coats lessened. Endothelial swelling continued taking on a syncitial appearance. The surface epithelium showed some necrosis and sloughing at the tips of the rugae. After 10 hours leukocytic infiltration of the tunica propria and submucosa were noted in addition to the above observations.

During the first 10 hours the heterophil was the prominent leukocyte, with relatively few lymphocytes present. It was noted that PAS staining sharply differentiated between heterophils and lymphocytes. Heterophil cytoplasm stained red-purple (with loss in intensity following sali-

vary digestion) while lymphocytes remained unstained.

Edema of the submucosa and muscle coats was still extensive after 24 hours. Blood and lymph vessel dilation continued, with margination of leukocytes. Fibroblasts remained abundant, but the granule cell layer regressed to only two cells deep.

Very prominent lesions, predominantly in the submucosa (Pl. I, Fig. 2), were noted after 24 hours. The largest of these lesions were tubercle-like structures similar to those associated with infectious granulomas. They consisted of a necrotic center surrounded by large immature cells, resembling phagocytic epithelioid cells, enclosed by fibroblasts and collagenic strands (Pl. I, Fig. 3). The presence of granule cells among the immature cells was not uncommon. Various stages in "tubercle" formation and degeneration were found, the later stage resembling the Langhans type of foreign body giant cell. Another prominent lesion derived from small blood vessels resulted from a fusion of swollen and degenerating endothelial cells. The lumina of these vessels were occluded by laminated concretions.

Over the 24-hour period the surface epithelium continued to be granular and vacuolated, while the serous cells returned to normal. By 48 hours the edema and congestion in the blood vessels had lessened and the tubercle-like lesions were less frequent. Areas of necrosis, predominantly infiltrated by lymphocytes, were prominent. Inflammation had greatly subsided after 60 hours. Return to normal was apparent in the stomach by 72 hours.

Lesions in the ascending intestine, although more marked than in the caeca, were less prominent than in the stomach. Intestinal and caecal epithelium exhibited some necrosis, accompanied by serosal edema, as early as one hour following turpentine injection. Increased liquefaction and leukocytic infiltration were evident after 3 hours and continued for the initial 6 hours. Necrosis was attributed to enzymatic digestion, particularly by pancreatic enzymes. Goblet cells in the intestines and caeca were greatly dilated after 7 hours and remained so for 48 hours. Granule cells and inflammatory cells were prominent after 7 hours, the number of granule cells remaining high over the entire 72-hour period.

Edema of the mucosal connective tissue and sloughing of the apical epithelium were extensive around 10 hours, with the swelling lasting the 3-day period. Heterophil infiltration of the necrotic areas was marked prior to 48 hours, followed by lymphocytes after the second day. Peritonitis was also evident. The stratum compactum of the digestive tract showed little response to the irritant during this time. By 72 hours more normal tissue architecture was evident, although edema and inflammation of the tunica propria persisted.

Turpentine caused destruction of fat and overlying pancreatic acini during the first 3 hours. Liquefaction and leukocytic invasion were marked by 10 hours. Thickening of the basement membranes in the larger Islets was the sole lesion noted in these areas. Although necrotic foci persisted, normal tubular structure was present in the pancreas at 60 hours.

Cortisone administered to trout previously injected with turpentine markedly altered the histological picture. Tissues excised 24 hours after concurrent injections of cortisone and turpentine did not exhibit the degree of lesions resulting from turpentine alone. The gastric lesions in these trout resembled those noted 7 hours after turpentine alone (Pl. I, Fig. 4). Granule cell number in these trout increased in the tunica propria and submucosa. Although submucosal edema occurred, minimal inflammatory response was found with concurrent injections. Tissues from trout given cortisone 24 hours prior to turpentine exhibited no apparent inflammatory response, although in control animals, which received cortisone alone, slight increases in granule cells were noted after 6 and 24 hours.

In the trout injected with Thorotrast 24 hours prior to turpentine, the distribution of Thorotrast after 30 hours was comparable to that found after 3 days in normal animals (Weinreb & Bilstad, 1955). In addition, a greater concentration of foreign matter was present in the blood and lymph vessels at this earlier time. Both the rate of pickup and amount of Thorotrast concentrated in the macrophages indicated increased phagocytosis coincident with inflammation. It was also noted that the loci of Thorotrast particles were rendered more visible after PAS staining.

Trauma produced by cutting the descending intestine did not appear to produce any noticeable effects on the trout behavior, and no mortalities resulted. On autopsy, however, gross differences were visible between cortisone-treated and untreated trout. In cortisone-treated trout the wound remained open, whereas in untreated animals the incision was difficult to find. In the latter group the cut area was hidden by thick exudate. Binding fibrinous exudate was lacking, or present to a lesser degree, after cortisone administration. Increased body fluid and signs of

inflammation were also more evident in the untreated group.

Tissue excised from the untreated trout 12 hours after operation exhibited acute inflammation, particularly in the tunica propria, and hyaline degeneration of the muscle coat near the cut. Tissue from the cortisone-treated group, excised after the same time interval, showed moderate inflammation with minimal hyperemia and granulation tissue formation. In addition, there was notable mucosal necrosis. Tissue removed after 24 hours showed comparable contrast. Intestine from untreated animals exhibited prominent granulation tissue in the mucosal folds (Pl. II, Fig. 5), whereas following cortisone no indication of healing was seen (Pl. II, Fig. 6). After 48 hours, granulation tissue was more extensive, some peritonitis was noted and there was an increase in granule cell number. In the one animal seen after 72 hours, tissue destruction and acute inflammation were extensive (Pl. II, Fig. 7); secondary infection was superimposed upon the original inflammation.

Discussion

Early signs of inflammation in the digestive organs were in direct response to absorption of the irritant from the body cavity. The speed of reaction was attributed to the rapidity of turpentine penetration and the physiological response of the trout. The characteristic signs of inflammation are directly associated with the presence of an irritant in the tissues; the turpentine, *per se,* does not directly initiate the reaction. Similar responses, using other irritants, were noted by Moon & Tershakovec (1951), who attributed this response to tissue chemotaxis.

Menkin (1940.2) previously had proposed that different factors released in the exudate stimulated leukocytosis (leukocytosis-promoting factor, LPF), followed by cell migration with increased capillary permeability (leukotaxine). He also reported a pH shift of the exudate from alkaline during the acute stage to acid in later stages. This pH shift is probably associated with the particular leukocytes present at that stage, namely polymorphonuclear or mononuclear leukocytes. Applying Menkin's concepts to the trout, the early responses of the tissues would be initiated by a chemotactic exudate followed by evidence of inflammation. This sequence was inhibited by cortisone.

Inhibition of inflammation by various steroid hormones, and ACTH, was reported by Menkin (1940.2, 1942, 1951.1, 1951.2), who later (1954) proposed that the anti-inflammatory mechanism acted on the cellular level at the site of inflammation. Thomas (1953) had earlier suggested that cortisone impaired the functioning of the reticulo-endothelial system.

The reaction of the trout stomach, seen 24 hours after concurrent injections of turpentine and cortisone, was unlike the response noted 24 hours after turpentine alone, resembling instead the responses seen 7 hours after turpentine. Similar delays in inflammatory response due to cortisone were reported by Michael & Whorton (1951) and Spain and co-workers (1952). Trout given cortisone 24 hours prior to turpentine exhibited complete inhibition of inflammation. It appears that the presence of the hormone before the onset of inflammation may successfully inhibit formation of chemotactic agents, and/or inhibit the ability of the reticulo-endothelial system to respond.

The response of the blood elements of the trout seen in the tissues during inflammation corresponds with the changes noted in the circulating leukocytes (Weinreb, 1958). The heterophilia characteristic of the early stages of inflammation is correlated with the prevalence of these cells in the tissues during the acute stage, and attributed to increased output of immature cells under the influence of LPF. Increase in lymphocytes in the tissues is correlated with their decrease in the circulation following augmented migration without corresponding lymphocyte production. In trout given cortisone such leukocytic infiltration was inhibited and, in addition, few macrophages were present in the tissues. Dougherty & Schneebeli (1950) reported less neutrophilic and macrophagic response in inflammation in cortisone-treated mice, while Rebuck & Mellinger (1953) reported similar effects following cortisone treatment in man; the latter group also suggested injury to the organ sources of the leukocytes and macrophages.

Cellular changes in the trout organs were notable in the macrophages, granule cells and fibroblasts. Increased macrophage activity was demonstrated by rapid uptake of Thorotrast. Gordon & Katsh (1949) reported a similar increase in phagocytosis of Thorotrast in rats subjected to starvation, such activity being reduced in animals with adrenal insufficiency and enhanced by administration of cortical hormone. A correlation between increased activity of the R-E elements with stress and adrenal cortical stimulation was suggested. The response of the rainbow trout to induced inflammation, and that of rats under inanition, is comparable and attribut-

able to adrenal cortical stimulation of the R-E elements.

The changing numbers of granule cells in the stomach and intestine during inflammation indicates a cyclic activity. Three hours after turpentine injection the number of cells in the cardiac stomach increases above normal and remains so for 24 hours, reaching a peak at 7 hours. This increase is not due to migration from other parts of the digestive tract, equivalent numbers being present throughout its length. The number of cells is also slightly higher after cortisone injection. This increase appears to be in direct response to the irritant, probably mediated by way of the adrenal cortex.

The drop in granule cell number at 24 hours is coincident with the appearance of the submucosal tubercle-like lesions. Although granule cells are noted among the immature cells and fibroblasts, they are not directly involved in tubercle formation. The exact role of these cells is still undetermined. As inflammation subsided at about 60 hours, the number of cells returned to normal or slightly above normal. In trout given cortisone concurrently with turpentine, this cyclic response is absent. In these fish, after 24 hours, the cell number is still above normal and no tubercles are present. It appears that cortisone delays the reaction, the 24-hour response being like that seen at 7 hours, or blocks the mechanism eliciting the granule cell decrease.

The tubercle-like lesions are due to cellular destruction following introduction of the irritant. The immature epithelioid-like cells, though of indefinite origin, may be associated with leukocyte response by way of a common stem cell present in the tissues and hemopoietic organs. Cell fusion, forming giant cells, is probably a stage in degeneration involving changes in the cell membrane and cytoplasm. The important factor in inflammation, in all animals, is probably not a specific lesion or particular site, but the response of connective tissue elements, in general, to the presence of an irritant, with resultant phagocytic activity.

The necrosis noted in the intestine, caeca and pancreas in the early stages of inflammation is attributed to enzymatic digestion of injured cells. The minor destruction seen at the base of the intestinal folds, compared to that at the apices, is understood in view of the greatly increased goblet cell activity in these areas. The minimal effect in the caeca, compared to that in the intestine, is due to the lesser concentration of enzymes in the appendices. It is, further, possible that the muscle sphincters at the caecal openings into the intestine were contracted, although no evidence of this was found in tissue sections.

After cutting the intestine a marked contrast is seen in wound healing between cortisone-treated and untreated trout. This is explained by the effect of the hormone on the connective tissue response to the trauma. The failure of the wound to close in the treated fish is correlated with the decrease in the fibrinous exudate. Spain *et al.* (1952) reported a drop in the level of circulating fibrinogen in cortisone-treated mice with turpentine-induced abscesses, and suggested that this lowered fibrinogen level was correlated with the scarcity of fibrin at the site of inflammation. Baker & Whitaker (1950) also noted delay in cutaneous wound closure in rats after topical application of adrenal cortical extract. These workers reported atrophy of collagen and impaired growth of granulation tissue with inhibition of fibroblast proliferation.

Inhibition of granulation tissue around turpentine abscesses after cortisone treatment has also been described by Shapiro and co-workers (1951), Taubenhaus *et al.* (1952) and Taubenhaus (1953). The effects of prolonged treatment of non-traumatized skin in rats given cortisone and compound F were noted by Castor & Baker (1950). Inhibition of fibroblast proliferation in rats following ACTH therapy was also reported by Baker & Ingle (1948). Similiar inhibition occurred in rats with turpentine abscesses after administration of testosterone propionate and estradiol dipropionate by Taubenhaus & Amromin (1949).

It therefore appears that cortisone, related steroids and ACTH retard wound healing by inhibition of connective tissue elements. It is also evident that the mechanism in the rainbow trout is similar to that reported for mammals.

Summary

1. Responses of digestive tract tissues were studied over a 72-hour period following turpentine injection and surgical trauma. Histological changes, characteristic of acute inflammation, were noted in the stomach, intestine and caeca one hour after injection, being most prominent in the cardiac stomach.
2. Increase in the number of fibroblasts and granule cells in the stomach was marked after 7 hours, followed by decrease in granule cell number at 24 hours with the appearance of "tubercles," returning to almost normal after 2 days. A cyclic activity of the granule cell is suggested.

3. Lesions in the intestine and caeca were less extensive; necrosis was attributed to pancreatic enzyme digestion.
4. Cortisone given concurrently with turpentine resulted in a delayed response, tissue removed after 24 hours resembling that seen 7 hours after turpentine alone. Following pretreatment with cortisone no lesions were apparent.
5. Increased phagocytosis coincident with inflammation was demonstrated after Thorotrast and turpentine injections. Augmentation of macrophagic activity was attributed to adrenal cortical stimulation of R-E elements with stress.
6. Cortisone injection preceding incising of the intestine resulted in inhibition of wound healing, with less granulation tissue, fewer inflammatory cells and more extensive necrosis.
7. The response of blood elements in the tissues corresponds with changes noted in the circulating leukocytes. The physiological responses in the rainbow trout are similiar to those reported in mammals under comparable conditions.

Literature Cited

Baker, B. L., & D. L. Ingle
1948. Growth inhibition in bone marrow following treatment with adrenocorticotropin (ACTH). Endocrinology, 43: 422-429.

Baker, B. L., & W. L. Whitaker
1950. Interference with wound healing by the local action of adrenocortical steroids. Endocrinology, 46: 544-551.

Cartwright, G. E., C. J. Grubler, L. D. Hamilton, N. M. Fellows & M. M. Wintrobe
1951. The role of the adrenal cortex in the regulation of the plasma iron level and the functional state of the reticulo-endothelial system. Proc. Second Clin. ACTH Conf., 1: 405-412. (J. R. Mote, Ed.) Blakiston, Philadelphia.

Castor, C. W., & B. L. Baker
1950. The local action of adrenocortical steroids on epidermis and connective tissue of the skin. Endocrinology, 47: 234-241.

Clark, E. R., & E. L. Clark
1920. Reactions of cells in the tail of amphibian larvae to injected croton oil (aseptic inflammation). Am. J. Anat., 27: 221-254.

Dougherty, T. F., & G. L. Schneebeli
1950. Role of Cortisone in regulation of inflammation. Proc. Soc. Exptl. Biol. Med., 75: 854-859.

Gordon, A. S., & G. F. Katsh
1949. The relation of the adrenal cortex to the structure and phagocytic activity of the macrophagic system. Ann. N. Y. Acad. Sci., 52: 1-30.

Menkin, V.
1933. A note on the mechanism of fixation in an area of sterile inflammation. Proc. Soc. Exptl. Biol. Med., 30: 1069-1076.
1940.1. Studies on inflammation. XVIII. On the mechanism of leucocytosis. Am. J. Pathol., 16: 13-32.
1940.2. Effect of adrenal cortex extract on capillary permeability. Am. J. Physiol., 129: 691-697.
1942. Further studies on effect of adrenal cortex extract and various steroids on capillary permeability. Proc. Soc. Exptl. Biol. Med., 51: 39-41.
1951.1. Effects of cortisone on the mechanism of increased capillary permeability to trypan blue in inflammation. Am. J. Physiol., 166: 509-517.
1951.2. Effects of ACTH on the mechanism of increased capillary permeability to trypan blue in inflammation. Am. J. Physiol., 166: 518-523.
1954. On the anti-inflammatory mechanism of hydrocortisone (compound F). Science, 120: 1026-1028.

Michael, M., Jr., & C. M. Whorton
1951. Delay of early inflammatory response by cortisone. Proc. Soc. Exptl. Biol. Med., 76: 754-756.

Moon, V. H., & G. A. Tershakovec
1951. The trigger mechanism of acute inflammation. Am. J. Pathol., 27: 693-695.

Rebuck, L. W., & R. C. Mellinger
1953. Interruption by topical cortisone of leucocytic cycle in acute inflammation in man. Ann. N. Y. Acad. Sci., 56: 715-732.

Robinson, H. J., & A. L. Smith
1953. The effect of adrenal cortical hormones on experimental infection. Ann. N. Y. Acad. Sci., 56: 757-763.

Shapiro, R., B. Taylor & M. Taubenhaus
1951. Local effects of cortisone on granulation tissue and the role of denervation and ischemia. Proc. Soc. Exptl. Biol. Med., 76: 854-857.

Spain, D. M., N. Molomut & A. Haber
1952. Studies of the cortisone effects on the inflammatory response. J. Lab. Clin. Med., 39: 383-389.

Taubenhaus, M.
1953. The influence of cortisone upon granula-

tion tissue and its synergism and antagonism to other hormones. Ann. N. Y. Acad. Sci., 56: 666-673.

Taubenhaus, M., & G. D. Amromin

1949. Influence of steroid hormones on granulation tissue. Endocrinology, 44: 359-367.

Taubenhaus, M., B. Taylor & J. V. Morton

1952. Hormonal interaction in the regulation of granulation tissue formation. Endocrinology, 51: 183-191.

Thomas, L.

1953. Cortisone and infection. Ann. N. Y. Acad. Sci., 56: 799-813.

Weinreb, E. L.

1958. Studies on the histology and histopathology of the rainbow trout, *Salmo gairdneri irideus*. I. Hematology: Under normal and experimental conditions of inflammation. Zoologica, 43: 145-154.

Weinreb, E. L., & N. M. Bilstad

1955. Histology of the digestive tract and adjacent structures of the rainbow trout, *Salmo gairdneri irideus*. Copeia, (3): 194-204.

Yokoyama, H. O.

1947. Studies on the origin, development, and seasonal variations in the blood cells of the perch, *Perca flavescens*. Unpublished Ph.D. thesis, University of Wisconsin, Madison.

EXPLANATION OF THE PLATES

The following figures were made from sections fixed in Helly's solution; all figures with the exception of Fig. 7, which was stained with PAS, were stained with hematoxylin-eosin-azure.

Plate I

Fig. 1. Cross-section of cardiac stomach 7 hours following turpentine injection, showing increase in granule cells (GC).

Fig. 2. Cross-section of cardiac stomach 24 hours following turpentine injection, showing inflammatory reaction in tunica propria and submucosa, with edema of the submucosa. Outlined area in submucosa encloses tubercle-like lesion shown at higher magnification in Fig. 3.

Fig. 3. High-power view of "tubercle," showing central necrosis, fusion of immature cells (I) and peripheral fibroblasts (F).

Fig. 4. Cross-section of cardiac stomach 24 hours following concurrent injections of turpentine and cortisone. Note lack of lesions and similarity to 7-hour reaction.

Plate II

Fig. 5. Section of descending intestine in region of cut 24 hours following operation. Note inflammation, bridging of cut and granulation tissue (G).

Fig. 6. Edge of cut in descending intestine 24 hours following operation and pretreatment with cortisone. Note less inflammatory response and necrosis (N).

Fig. 7. Mucosa of descending intestine 72 hours following operation, showing cellular response to secondary infection. Heterophil, H; fibroblast, F.